Immunization with a DNA Plasmid Encoding the SAG1 (P30) Protein of *Toxoplasma gondii* Is Immunogenic and Protective in Rodents

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Immunization with DNA can induce humoral and cell-mediated immune responses, both of which are important in conferring immunity to *Toxoplasma gondii.* The efficacy of genetic vaccination with a cDNA encoding the *T. gondii* SAG1 (P30) surface antigen was evaluated. Sera of immunized mice showed recognition of *T. gondii* tachyzoites by immunofluorescence and exhibited high titers of antibody to SAG1 by ELISA. SAG1-stimulated splenocytes from vaccinated mice produced primarily interferon-γ and interleukin-2. Vaccinated mice survived challenge with 80 tissue cysts of ME49 strain, whereas all control mice died; challenge with 20 tissue cysts resulted in fewer brain cysts, compared with controls. Challenge of vaccinated rats with VEG strain oocysts resulted in a reduction in brain cysts. No protection was observed when mice were challenged with the highly virulent RH strain tachyzoites. These results suggest that nucleic acid vaccination can provide protection against *T. gondii* infection in mice.

Toxoplasma gondii is an intracellular parasite capable of infecting a variety of mammals and birds [1]. Although infection of immunocompetent humans is usually asymptomatic, toxoplasmosis still presents serious medical problems. Infection during pregnancy can result in neurologic and ocular complications in the fetus. Of even greater significance since the emergence of the AIDS pandemic is toxoplasmosis in the immunocompromised host. The most serious problem in these persons is the recrudescence of latent infection, often resulting in a fatal encephalitis [2]. Toxoplasmosis also causes mortality in animals, especially sheep and goats, among which infection during pregnancy often results in abortion [1].

Toxoplasmosis can be effectively treated with the combination of pyrimethamine plus sulfadiazine. However, there is a significant occurrence of adverse reactions to this therapy, especially in human immunodeficiency virus–infected patients, which often results in discontinuation of therapy and relapse of disease [3]. In pregnant women, serologic screening is necessary to identify newly acquired infection, because primary infection is largely asymptomatic and therapy with pyrimethamine must be avoided during the first 16 weeks because of potential teratogenicity. These considerations are compelling arguments for the development of a vaccine against toxoplasmosis. A live vaccine for sheep based on the attenuated S48 strain has been licensed in Europe and New Zealand [4]; however, concern that it might revert to a pathogenic strain makes it a poor vaccine candidate for humans.

Attempts to develop a subunit-based vaccine to *T. gondii* have focused on the major surface antigen SAG1 (P30). These studies have used purified SAG1 [5–7], recombinant SAG1 produced in *Escherichia coli* [8] or yeast [9], and SAG1-derived peptides [10, 11]. All of these studies have been encouraging, in that they have demonstrated development of significant protection in animal models. As an extension of these efforts, we have focused on the development of a DNA-based vaccine, because such vaccines have been shown in other systems to elicit strong humoral and cell-mediated immunity [12–14]. The use of plasmid DNA has been shown to afford protection against intracellular parasites such as *Plasmodium* [15] and *Leishmania* species [16]. We describe here the development and evaluation of a DNA vaccine based on a plasmid encoding SAG1 for control of toxoplasmosis.

Materials and Methods

Parasites. Three strains of *T. gondii* (RH [17], ME49 [18], and VEG [19]) were used. The RH strain is highly virulent for mice; it was used to derive the SAG1 cDNA clone [20] and to challenge mice. ME49 was used to challenge mice. This strain was selected because it produces many tissue cysts in the brain and is mildly virulent for mice. The VEG strain was used to challenge rats. RH and ME49 tachyzoites were maintained by passage in normal human fibroblasts. Brain tissue cysts of ME49 were obtained by passage through C57BL/6 mice and maintained by passage of 20 tissue cysts administered by intraperitoneal injection.

Plasmid construction. In intact *T. gondii,* SAG1 mRNA is first translated with a signal peptide of 30 amino acids [20] and then

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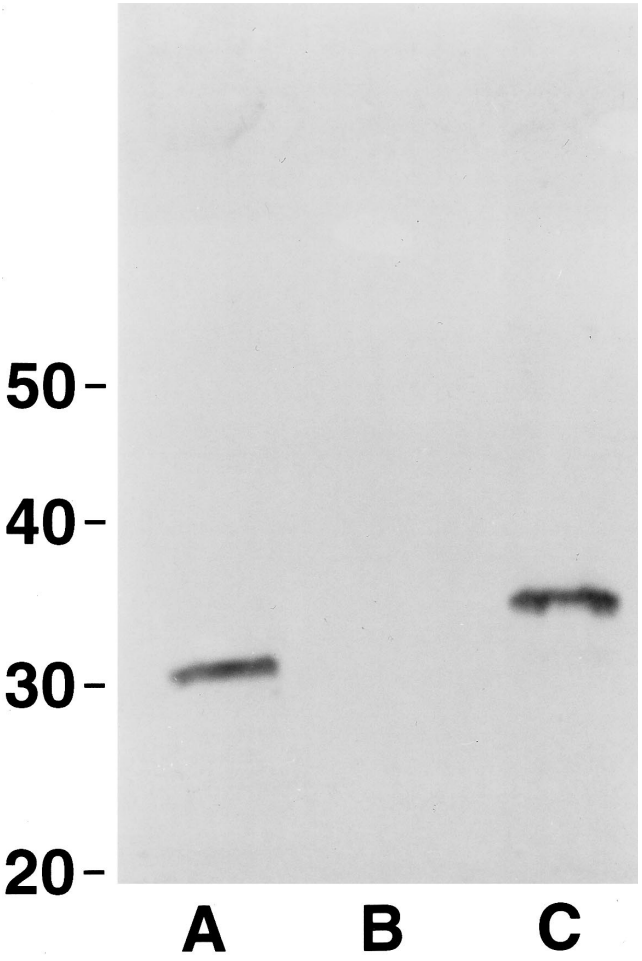

Figure 1. Western blot analysis under nonreducing conditions of recombinant SAG1 protein of *Toxoplasma gondii* expressed in rhabdomyosarcoma cells. Membrane was probed with SAG1-specific monoclonal antibody 213-17999 and developed with horseradish peroxidase–conjugated donkey anti-mouse antibody. Lane A, native RH strain SAG1; lane B, filtered medium from transfected cells; lane C, cell pellet from washed monolayer. Molecular weight markers (in kDa) of reduced proteins are shown at left.

processed to the mature form, which is bound to the membrane with a glycosyl-phosphatidylinositol anchor [21, 22]. The expression vector used in these studies, pCMVInt (provided by Vical, San Diego), contains sequences coding for the leader sequence of human tissue plasminogen activator (TPA). To avoid a potential problem from the presence of two tandem signal sequences, the signal sequence derived from SAG1 was eliminated during the construction of the recombinant plasmid pCMVToxo. A *Bam*HI fragment, coding for the carboxyl-terminal 291 amino acids of SAG1 but lacking the sequence coding for the signal peptide, was excised from a plasmid (gift of John Boothroyd, Stanford University, Stanford, CA) and ligated into pCMVInt, in frame with the sequence coding for the signal sequence of human TPA [23]. Expression of the resulting TPA-SAG1 fusion protein was under the control of the cytomegalovirus (CMV) immediate early promoter. The resulting plasmid, pCMVToxo, was purified by double-banding on CsCl. Sequence was verified by cycle sequencing by means of an ABI 373 instrument (Perkin-Elmer, Foster City, CA). The plasmid encoding murine granulocyte-macrophage colony–stimulating factor (pGM-CSF) was a gift of Stephen Johnston (University of Texas Southwestern Medical Center, Dallas, TX) and was purified by double-banding on CsCl.

Transfection of human rhabdomyosarcoma (RD) cells. RD cells (American Type Culture Collection, Manassas, VA) were grown to 50% confluence at 37°C in a humidified 5% CO_2 atmosphere in 35-mm wells in Dulbecco's modified Eagle medium containing 100 U/mL each penicillin and streptomycin and 10% fetal calf serum and were transfected with 2 μg of pCMVToxo or pCMVInt with 6 μL of Fugene (Boehringer Mannheim, Indianapolis). After 2 days, medium was collected and filtered through a 0.2-μm filter; cell monolayers were washed 3 times with 5 mL of PBS and then scraped into 1 mL of PBS. Cells were then pelleted by centrifugation at 13,000 *g* for 15 min. Cell pellets and medium were stored at −20°C until use.

Western blot analysis. Western blot analysis of transfected RD cells was done on cell pellets of single 35-mm wells. The pellet was boiled in 0.5 mL of Laemmli sample buffer (without reducing agent), and 20 μL was loaded onto a 12% polyacrylamide gel. After electrophoresis, the gel was transferred to a nitrocellulose membrane that was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS). The membrane was probed with a 1 : 1000 dilution of SAG1-specific monoclonal antibody 213-17999 (OEM Concepts, Toms River, NJ). Development was with horseradish peroxidase (HRP)–conjugated donkey anti-mouse antibody. For Western blot analysis of ME49 tachyzoites, ~20,000 tachyzoites were loaded onto each lane. Electrophoresis and transfer were as described above, and the membrane was probed either with monoclonal antibody 213-17999 (1 : 1000 dilution) or with 1 : 250 dilutions of sera from mice. Sera were obtained from mice 3 weeks after the second booster injection.

Endoglycosidase F analysis. Each cell pellet from a single well was suspended in 50 μL of denaturation buffer (Boehringer Mannheim) without β-mercaptoethanol and incubated at 95°C for 5 min. Next, 100 μL of reaction buffer (Boehringer Mannheim) was added, followed either by 5 μL (10 U) of endoglycosidase F or by 5 μL of water for the control reaction. After incubation at 37°C for 1 h, 15 μL of the reaction mixture was analyzed as described above.

Immunization. Six-week-old C57BL/6 female mice (10/group) were obtained from the Division of Cancer Treatment, National Cancer Institute (Frederick, MD). Intramuscular immunizations into the hindquarters were done with 100 μg of pCMVToxo or pCMVInt in 100 μL of PBS with 25% sucrose. All injections, for both vaccinated and control mice, also contained 50 μg of pGM-CSF, because of preliminary studies demonstrating improved immune response. Mice were boosted with the same dose at 3 and 6 weeks after the first injection. From selected animals, sera were obtained by terminal exsanguination 2 weeks after the final injection. For immunizations with purified SAG1 protein, SAG1 was purified as described by Debard et al. [5] from RH-strain tachyzoites commercially obtained from OEM Concepts, and 50 μg of purified protein in 0.1 mL of PBS was mixed with an equal volume of Freund's complete adjuvant and injected into 5 mice at several locations at the base of the tail. Booster injections were done in a

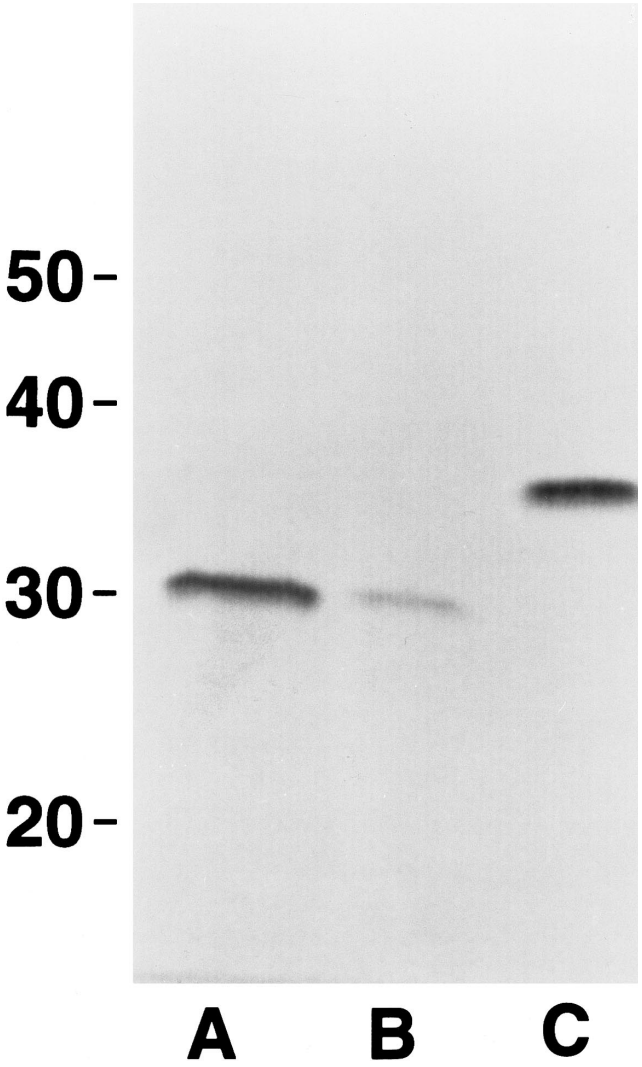

Figure 2. Western blot analysis of endoglycosidase F treatment of recombinant SAG1 protein of *Toxoplasma gondii* expressed in rhabdomyosarcoma cells. Membrane was probed with SAG1-specific monoclonal antibody 213-17999 and developed with horseradish peroxidase–conjugated donkey anti-mouse antibody. Lane A, native SAG1; lane B, cell pellet treated with endoglycosidase F; lane C, cell pellet without endoglycosidase F treatment.

similar manner 3 and 6 weeks after the first injection, but Freund's incomplete adjuvant was substituted.

Eight-week-old Sprague-Dawley female rats were obtained from Taconic Farms (Germantown, NY). Intramuscular immunization (5 rats/group) was done as for the mice but with booster administration at 4 and 8 weeks. Sera were obtained by retroorbital puncture 2 weeks after the final injection.

ELISA. For ELISA, 96-well microtiter plates were coated with 20 ng of native, immunopurified SAG1 per well. Mouse sera were diluted in PBS and applied to the wells, followed by goat anti-mouse IgG–HRP conjugate as secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA). For isotype analysis, mouse sera, diluted 1 : 100 in PBS, were applied to the plates and developed with goat anti-mouse IgG1– or IgG2a–HRP conjugate (Boehringer Mannheim). Rat sera, diluted 1 : 250 in PBS, were applied to the plates and developed with goat anti-rat IgG–HRP conjugate. Sera from vaccinated animals were considered to be positive if the mean value of triplicate determinations was >3 SD above the mean value obtained with sera from control animals.

Immunofluorescence. ME49 tachyzoites were fixed onto slides with cold methanol, exposed to sera (at a 1 : 100 dilution in 5% goat serum in TBS) for 1 h, and washed, and fluorescence was developed by the addition of fluorescein-labeled goat anti-mouse secondary antibody (Jackson Immunoresearch Laboratories).

Measurement of cellular immune responses. Spleens were aseptically removed from mice 3 weeks after the last booster injection, and single-cell preparations were made as described elsewhere [24]. Cells (2×10^5/well) were plated in RPMI with 10% fetal calf serum onto 96-well microtiter plates. One microgram of purified SAG1 was added to each well, and cultures were maintained in 5% CO_2 for 48 h. Cytokines released into the medium were quantified by ELISA (R&D Systems, Minneapolis). Three mice were used for each set. Data are expressed as means ± SDs.

Challenge. For challenge studies that used *T. gondii* ME49 strain [18], mice were administered ~80 tissue cysts via gastric gavage 8 weeks after the first immunization. For studies involving the determination of tissue-cyst burden in the brain, mice were given 20 ME49 strain tissue cysts via gavage. Brains were removed from mice 6 weeks after infection, placed in 1 mL of PBS, and homogenized by repeated passage through a 19-gauge, followed by a 21-gauge, needle. Tissue cysts were enumerated by counting 10-μL aliquots under a microscope at ×100 magnification. Tissue cysts were counted in 3 separate aliquots for each brain. Challenge studies of mice that used RH-strain tachyzoites were done by intraperitoneal injection of ~1000 tachyzoites.

Rats were challenged 122 days after the first immunization by oral administration of 10,000 VEG strain oocysts as described elsewhere [19] and were killed 123 days after challenge. From each rat,

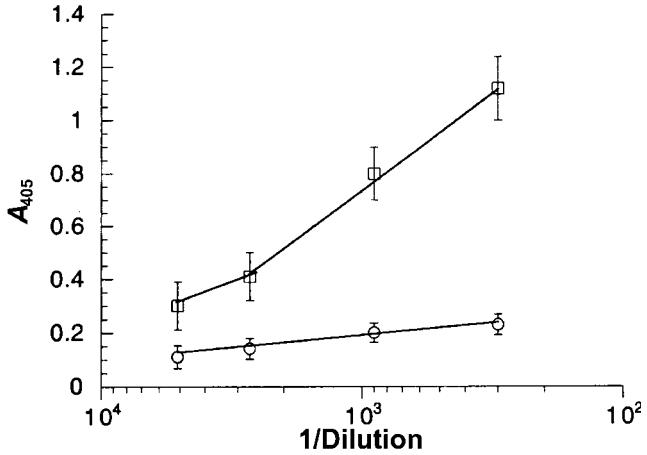

Figure 3. Antibody titers of mice injected either with recombinant plasmid pCMVToxo (□) or with control plasmid pCMVInt (○). Data are absorbance at 450 nm and are means from 5 mice. Error bars indicate SDs.

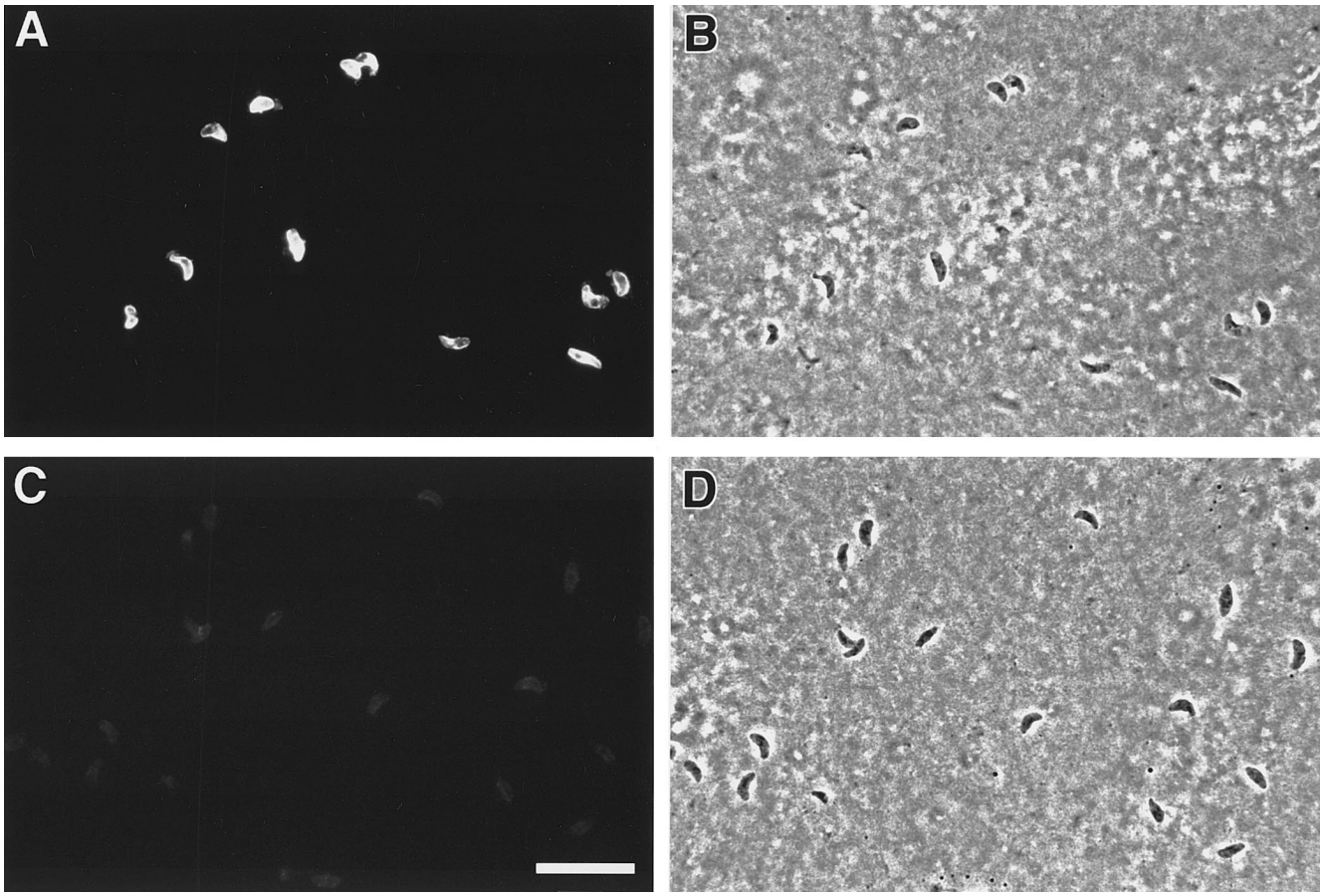

Figure 4. Immunofluorescence of ME49 tachyzoites probed with sera (1 : 100 dilution) from mice after 3 immunizations with recombinant plasmid pCMVToxo (*A*) or control plasmid pCMVInt (*C*). *B, D,* phase-contrast photomicrographs of *A* and *C,* respectively. Bar = 20 μm. Magnification, ×1000.

one-half brain was homogenized in 2 mL of saline (total volume, 2.5 mL), and tissue cysts were counted in 100 μL of the brain homogenate.

Statistical analysis. Results were analyzed by Student's *t* test by means of Systat software (SPSS, Chicago).

Results

In attempting to develop a DNA vaccine, we have focused on the gene encoding SAG1, because previous studies for preventing toxoplasmosis have shown that immunization with SAG1 peptides or protein can induce protection. To verify that the plasmid construct pCMVToxo was capable of expressing SAG1 in muscle cells, it was transfected into RD cells, a human RD line. Western blot analysis of both the medium from the transfected monolayers and the cells indicated that a protein of ~34 kDa was recognized in the cellular fraction by 213-17999, a monoclonal antibody specific for SAG1 (figure 1, lane C). No immunoreactive material was found in the filtered medium of the monolayers (figure 1, lane B), indicating that the recombinant material remained cell-associated and was not secreted into the medium. No immunoreactive material was detected in either the cell pellet or the medium of cells transfected with the expression vector pCMVInt alone (data not shown).

The apparent molecular weight of the recombinant protein produced in the transfected cells was greater than that of native SAG1, as detected in tachyzoites (figure 1, lanes A and C). This finding is consistent with results of Kim et al. [25] and Biemans et al. [9], who reported that recombinant SAG1 produced in Chinese hamster ovary (CHO) cells exhibited a higher apparent weight than did the tachyzoite protein. In their case, the increase in size was attributable to *N*-glycosylation. To determine whether the recombinant SAG1 produced in the RD cells was *N*-glycosylated, it was subjected to treatment with endoglycosidase F. Figure 2, lane B, shows the results of Western blot analysis of the recombinant SAG1 after treatment with endoglycosidase F. The apparent molecular weight of the recombinant protein after endoglycosidase F treatment was indistinguishable from that of native SAG1, indicating that the increase in size of the recombinant protein was due to *N*-glycosylation.

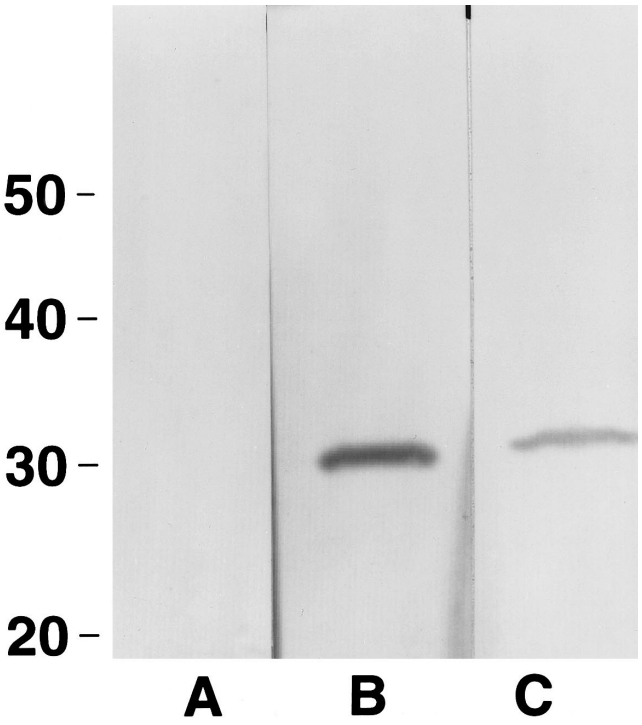

Figure 5. Western blot analysis of ME49 tachyzoites. Lane A, membrane probed with sera from mice injected with control plasmid pCMVInt; lane B, membrane probed with sera from mice injected with recombinant plasmid pCMVToxo; lane C, membrane probed with SAG1-specific monoclonal antibody 213-17999. Membranes were developed with horseradish peroxidase–conjugated donkey anti-mouse antibody. Molecular weight markers (in kDa) of reduced proteins are shown at left. CMV, cytomegalovirus.

To examine the vaccine potential of pCMVToxo, mice were immunized intramuscularly with pCMVToxo plus pGM-CSF (vaccinated) or with pCMVInt plus pGM-CSF (control). Blood samples were obtained after the immunization schedule was completed, and antibody titers were determined by ELISA, with purified SAG1 as the bound target. Figure 3 shows that a strong response was elicited by the immunization protocol, with detectable titers at up to a 1 : 5400 dilution of the immune sera. All sera obtained from vaccinated rats were positive at a dilution of 1 : 250.

A comparison of the deduced amino acid sequences of the mature SAG1 protein of the RH and ME49 strains indicates that there are seven differences [6]. Because the cDNA clone used in the construction of pCMVToxo was derived from the RH strain of *T. gondii,* it was necessary to verify that immune responses generated following immunization were capable of recognizing the SAG1 found on the surface of tachyzoites of ME49, the strain to be used in the subsequent challenge studies. To test the ability of sera from mice immunized with the RH-based plasmid to recognize ME49 tachyzoites, we assayed isolated tachyzoites of the ME49 strain by immunofluorescence (figure 4). A strong pattern of fluorescence was observed when the tachyzoites were probed with sera obtained from mice injected with pCMVToxo, compared with that seen with sera from animals injected with the control plasmid. These results thus indicate that the sera from mice vaccinated with a plasmid encoding for the SAG1 sequence found in the RH strain are able to recognize the protein expressed on the surface of ME49 tachyzoites. To demonstrate that the response was mounted only against the SAG1 protein, Western blot analysis was done. As shown in figure 5, antisera from mice injected with pCMVToxo (lane B) were immunoreactive with a protein of ~30 kDa, identical in size to protein immunoreactive with monoclonal antibody specific for SAG1 (lane C). Antisera from control mice injected with pCMVInt (lane A) did not develop this pattern.

To evaluate whether the T helper involvement resulting from the DNA vaccine was primarily Th1 or Th2 type, we determined the levels of IgG1 and IgG2a in mice immunized either with pCMVToxo or with purified SAG1 administered with Freund's adjuvant. Mice immunized with pCMVToxo showed a mean (±SD) ratio of IgG1 to IgG2a of 0.81 ± 0.29. In contrast, mice immunized with purified SAG1 showed a ratio of 8.9 ± 2.8, implying that vaccination with the plasmid offers a better Th1-type response than does vaccination with the protein.

A second approach to determine the T helper type of response is to measure the cytokines produced by splenocytes on antigenic stimulation. Cultured splenocytes from vaccinated animals demonstrated a preferential production of interferon-γ and interleukin-2 on stimulation with purified SAG1, with little production of interleukin-4, suggesting that the response was oriented to a Th1 type (table 1).

Since DNA immunization induced both humoral and cell-mediated immune responses that could potentially provide protection against toxoplasmosis, we performed challenge studies of mice and rats. Figure 6 is a Kaplan-Meier plot comparing survival of vaccinated and control mice after peroral challenge with 80 ME49 tissue cysts. All 10 mice in the vaccinated group survived, whereas all 10 of the control animals died within 8 days of challenge. This experiment was repeated, resulting in the survival for 30 days of 9 of 10 mice in the vaccinated group; no mice in the control group survived past 10 days (data not

Table 1. Cytokine production by splenocytes of immunized mice after antigenic stimulation.

	Treatment		
Cytokine	pCMVInt	pCMVToxo	20 *Toxoplasma gondii* cysts
Interferon-γ	<2[a]	129 ± 42	503 ± 129
Interleukin-4	<2[a]	<2[a]	79 ± 28
Interleukin-2	<4[a]	278 ± 89	489 ± 36

NOTE. Splenocytes were obtained from 5 mice in each group 2 weeks after final injection of plasmid or 4 weeks after gavage with *T. gondii* cysts. Data are pmol/mL, mean ± SD, unless otherwise indicated. CMV, cytomegalovirus.

[a] Below threshold of sensitivity of assay.

Table 2. Enumeration of *Toxoplasma gondii* tissue cysts in brains of vaccinated and control mice and rats.

Immunization	Mice (n = 10)	Rats (n = 5)
pCMVInt	1244 ± 367	200 ± 22
pCMVToxo	362 ± 104[a]	80 ± 41[a]

NOTE. Mice and rats were immunized with recombinant plasmid pCMVToxo or control plasmid pCMVInt. Data are cysts per brain, mean ± SD. CMV, cytomegalovirus.

[a] $P < .01$ vs. control.

shown). When mice were challenged by intraperitoneal injection of tachyzoites from the highly virulent RH strain, however, no differences were noted in the survival of the groups, with all animals dying within 8 days.

In addition to determining whether it is possible to produce protection against mortality from acute toxoplasmosis, we were interested in determining whether the vaccination would provide any protection against the formation of *T. gondii* tissue cysts in the brain. For these experiments, mice and rats were vaccinated as described, but mice were challenged with a lower dose of tissue cysts, with peroral administration of 20 ME49 tissue cysts. Although rats were coinjected with the plasmid pGM-CSF, which encodes the murine cytokine, there is evidence that it is active in rat tissue [26]. In these experiments, all mice in both control and vaccinated groups survived for 6 weeks, and all rats survived for 123 days. At that time, the animals were killed and their brains removed for enumeration of *T. gondii* tissue cysts (table 2). There was a decrease in tissue cysts in both rats and mice in the vaccinated groups, compared with control groups. Analysis of these data by the independent t test indicated that they were significantly different ($P < .01$).

Discussion

This study has shown that a DNA vaccine encoding the SAG1 protein of *T. gondii* can elicit a broad range of immune responses that are capable of decreasing mortality of animals acutely infected with *T. gondii.* It also demonstrates that the DNA vaccine is capable of reducing the levels of tissue cysts in the brains of infected animals.

Transfection of human RD cells with pCMVToxo resulted in the production of a single protein that was immunoreactive on Western blot analysis with SAG1-specific monoclonal antibody 213-17999 (figure 1). The recombinant SAG1 protein is found only in the cell monolayer and not in the filtered culture medium, indicating that it was not processed through a secretory route. The apparent molecular mass of the recombinant protein, as determined by Western blot analysis, of nonreduced samples is 34 kDa, larger than that of the mature protein isolated from *T. gondii* tachyzoites. This increase in apparent molecular weight of the recombinant forms of SAG1 has been observed in both CHO cells and in *Pichia* organisms. Treatment of the recombinant SAG1 protein with endoglycosidase F, which removes *N*-linked oligosaccharides, resulted in the generation of a protein with an apparent molecular weight indistinguishable from that of the protein found in tachyzoites (figure 2). These results are consistent with those of Kim et al. [25] and Biemans et al. [9], who demonstrated that the increase in size of the recombinant protein was due to *N*-linked glycosylation.

Intramuscular immunization of mice resulted in the development of high titers (>1 : 5000) of SAG1-specific antibody (figure 3), as determined by ELISA with purified SAG1. All immunizations were done by coinjecting murine pGM-CSF. A number of investigations have established that coinjection of murine pGM-CSF affords adjuvant effects for DNA vaccines [27, 28], and coinjection has been shown to increase the protection afforded by a malaria DNA vaccine [29]. In preliminary studies, we found that including pGM-CSF increased antibody titers, and thus it was included in the immunization protocol.

The antibodies induced by immunization with an RH strain–derived gene recognize SAG1 on the surface of ME49 strain *T. gondii* tachyzoites (figure 4), and immunization protected against subsequent infection with the ME49 and VEG strains. Thus, the immune responses that are induced are not strain-specific, which is an important characteristic for a vaccine candidate.

One of the goals of a vaccination protocol is to be able to appropriately direct the T helper response. For *T. gondii,* it has been demonstrated that a Th1-biased response is the result of a naturally occurring infection [30]. Thus, it would appear that a vaccination protocol that directs a Th1 rather than a Th2 response is desirable. We evaluated the T helper response of vaccinated mice by two methods. The first method was to determine the ratio of IgG1 to IgG2a. Mice exhibit a preferential

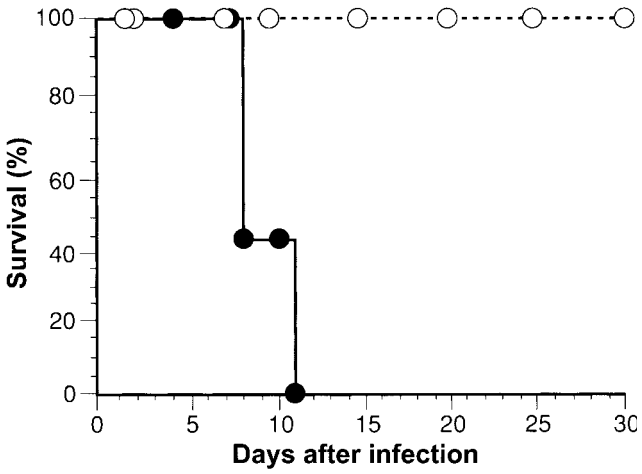

Figure 6. Kaplan-Meier curve for survival in mice immunized with recombinant plasmid pCMVToxo (○) or control plasmid pCMVInt (●) and challenged with ME49 tissue cysts of *Toxoplasma gondii.* Two weeks after final injection, mice (10 per group) were administered by gavage ~80 tissue cysts of ME49 strain freshly isolated from brains of C57BL/6 mice. CMV, cytomegalovirus.

production of IgG2a when the response is Th1-driven [31]. As shown in the Results section, DNA vaccination with p-CMVToxo leads to a low ratio of IgG1 to IgG2a, whereas immunization with purified SAG1 leads to a predominantly IgG1 response. This indicates that the response is oriented toward a Th1-type response when vaccination is done with a plasmid versus a Th2-type response when the purified protein is administered. These results are consistent with those of Petersen et al. [32], who found that vaccination with a recombinant SAG1 expressed in *E. coli* yielded a primarily Th2-type response. The results of the cytokine production by in vitro–stimulated splenocytes (table 1) supports the conclusion that vaccination with pCMVToxo preferentially induces a Th1 response. Both interferon-γ and interleukin-2 were produced on stimulation of splenocytes from immunized mice, whereas there was no detectable production of interleukin-4, consistent with a Th1-type response. These findings were encouraging, because it has been shown that interferon-γ production is important in the control of infection by *T. gondii* [30, 33].

Challenge of C57BL/6 mice with a lethal dose of tissue cysts of the moderately virulent ME49 strain gave a conclusive indication that DNA vaccination could afford protection against acute toxoplasmosis, because none of the vaccinated mice died. It is noteworthy that the current vaccine did not protect against intraperitoneal challenge with the RH strain of *T. gondii.* This suggests that the immune response induced by immunization is not as broad or as intense as that provided by infection with an attenuated strain of *T. gondii,* such as ts-4 [34]. Since other studies have demonstrated partial protection after immunization with SAG1 protein [6], it is possible that boosting with native or recombinant SAG1 may enhance the protective ability of polynucleotide vaccination. Alternatively, it may be necessary to alter the dose, timing, or route of administration of the plasmid to optimize the appropriate response or to use cDNA constructs that encode sequences for additional *T. gondii* proteins. A further validation of the efficacy of DNA vaccination is found in the comparison of the number of brain tissue cysts in vaccinated and control animals (table 2). Although the vaccination did not completely eliminate tissue cysts in the animals, it did lead to a substantial reduction in the cyst burden. In addition, this reduction shows that a SAG1-derived construct is effective in vivo against at least two strains of *T. gondii,* ME49 and VEG, derived from different clonal lineages [35], and against infections initiated by two different stages of the parasite (bradyzoites and sporozoites). The results obtained from the rats also demonstrate that this method of vaccination is effective in two species of hosts.

In summary, the use of a plasmid DNA encoding the SAG1 of *T. gondii* offers a promising approach as a vaccine against toxoplasmosis. Evaluation in additional species, including those in which a vaccine may have veterinary utility, should help define the role that this vaccine may play in preventing toxoplasmosis. Development of DNA vaccines against toxoplasmosis that use other *T. gondii* proteins, including bradyzoite-specific proteins, may also lead to potentially useful vaccines.

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